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14. ABSTRACT
About one out of every ten cases of epithelial ovarian cancer (EOC) is inherited. The majority, >90%, of inherited cases of EOC are the result of mutations in the breast cancer associated gene 1 (BRCA1). This gene was originally identified based on genetic linkage to families with an increased risk of developing breast and ovarian cancer. It is involved in controlling normal cellular growth and is thought to suppress the growth of tumors. That is, if BRCA1 is mutated, the risk to develop breast and ovarian cancer increases. Another gene that is important in the development of cancer is p53. It also helps maintain normal cellular growth and is the most commonly mutated gene in all human cancers. The p53gene has been shown to be mutated in at least 50% of all cases of epithelial ovarian cancer. In addition to mutations of BRCA1, mutations of the p53gene are often found in patients with breast and ovarian cancer syndrome. Based on the importance of both of these genes in the development of this type of ovarian cancer, we hypothesize that inactivation of BRCA1 and p53in the ovaries of mice will result in epithelial ovarian cancer in the animals.

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MODELING HUMAN EPITHELIAL OVARIAN CANCER IN MICE BY ALTERATION OF EXPRESSION OF THE *BRCA1* AND/OR *P53* GENES

PI: Denise C. Connolly
Progress Report, February 19, 2007

Introduction:

About one out of every ten cases of epithelial ovarian cancer is inherited. Unlike non-hereditary (sporadic) ovarian cancer, some of the underlying genetic causes of hereditary ovarian cancer are well understood. The majority, >90%, of inherited cases are the result of inherited mutations in the breast cancer associated gene 1 (*BRCA1*). This gene was originally identified based on genetic linkage to families with an increased risk of developing breast and ovarian cancer. It is involved in controlling normal cellular growth and is thought to suppress the growth of tumors. That is, if BRCA1 is mutated, the risk to develop breast and ovarian cancer increases. Another gene that is important in the development of cancer is the *p53* gene. It also helps maintain normal cellular growth and is the most commonly mutated gene in all human cancers. It has been shown to be mutated in at least 50% of all cases of epithelial ovarian cancer. In addition to mutations of *BRCA1*, mutations of the *p53* gene are often found in patients with breast and ovarian cancer syndrome. Based on the importance of both of these genes in the development of this type of ovarian cancer, we hypothesize that inactivation of *BRCA1* and *p53* in the ovaries of mice will result in epithelial ovarian cancer in the animals.

The objectives of this funded proposal are to:

1. develop mouse models of human epithelial ovarian cancer by inactivation of BRCA1 and p53 singly or at the same time in the mouse ovarian surface epithelial cells;
2. investigate whether there is a difference between the complete absence of p53 or the presence of a dominantly acting p53 mutant in ovarian tumorigenesis in mice; and,
3. identify genes and cellular pathways, downstream of BRCA1 and p53 inactivation/mutation, that contribute to ovarian carcinogenesis.

Body:

Specific tasks that were completed during months 24-36 of the funding period include; 1) completion of breeding of the mice necessary for the study, 2) completion of intrabursal Adenovirus-Cre recombinase injection in all study groups, and 3) evaluation of tumor formation in mice. All mouse procedures described in this progress report were approved by Fox Chase Institutional Animal Care and Use Committee.

As stated in the 2006 progress report, we obtained superior results using intrabursal injection of Ad5-CMV-Cre for delivery of Cre recombinase to the ovarian epithelium. Based on these findings, all mice harboring floxed alleles of BRCA1 and/or p53 were subjected to intrabursal injections of Ad5-CMV-Cre as summarized in Table 1 below. A total of five groups of mice with the genotypes $BRCA1^{LoxP/LoxP}$, $p53^{LoxP/LoxP}$, $BRCA1^{LoxP/LoxP}/p53^{LoxP/LoxP}$, $BRCA1^{LoxP/LoxP}/p53^{R172H/WT}$ and $BRCA1^{LoxP/LoxP}/p53^{LoxP/R172H}$ were subjected to intrabursal Ad5-CMV-Cre injection and evaluated for ovarian tumor formation.

Table 1. Summary of Ad5-CMV-Cre injections in mice bearing LoxP flanked alleles of *BRCA1* and/or *p53*.

	Group I	**Group II**	**Group III**	**Group IV**	**Group V**
Injection	**$BRCA1^{LoxP/LoxP}$**	**$p53^{LoxP/LoxP}$**	**$BRCA1^{LoxP/LoxP}$ $p53^{LoxP/LoxP}$**	**$BRCA1^{LoxP/LoxP}$ $p53^{WT/R172H}$**	**$BRCA1^{LoxP/LoxP}$ $p53^{LoxP/R172H}$**
Bilateral Ad5-CMV-Cre	**44**	**41**	**42**	**41**	**26**
Single ovary Ad5-CMV-Cre	**6**	**6**	**6**	**6**	**6**
PBS	**6**	**6**	**6**	**6**	**6**

At the time of submission of this progress report 161 mice from the five study groups have been euthanized. Animals were euthanized if they were moribund or because they had reached old age without any symptoms of disease. In the absence of symptoms of disease, most animals were euthanized by the time they reached ~500 days in age. In rare cases animals that had expired overnight or over weekends were considered not suitable for tissue collection and further evaluation which accounts for small differences in the total numbers of mice injected versus the total number of mice evaluated. A summary of the total number of animals in each group that were evaluated is presented in Table 2. All animals were euthanized by CO_2 asphyxiation and were subjected to complete necropsy with gross pathological evaluation. All reproductive tract tissues and other affected tissues were removed and either fixed and processed for paraffin embedding and tissue sectioning or snap frozen in liquid nitrogen. Paraffin embedded tissue sections of reproductive tracts and all affected tissues were stained with hematoxylin and eosin (H&E) and evaluated microscopically by the PI (D. Connolly) and by Dr. Lora Hedrick Ellenson, a board certified Gynecological Pathologist at Weill Cornell Medical College in New York. Paraffin embedded tissues of a number of tumors and affected tissues were also subject to immunohistochemical staining with antibodies to detect various cellular markers of differentiation (e.g., cytokeratin 8, cytokeratin 19, CD3, CD45 and α-smooth muscle actin) to assist in diagnosis of tumor histopathology. To confirm that tumor formation observed in mice is attributable to loss of *BRCA1* and/or *p53* expression, tumor specimens were analyzed for excision of loxP flanked (floxed) sequences by PCR of genomic DNA isolated from tumor specimens. Genomic DNA from tumor specimens was isolated from snap frozen tumor specimens for large tumor specimens or by manual microdissection of neoplastic lesions identified in paraffin embedded and sectioned tissues. A total of 161 mice were euthanized and evaluated for tumor formation (Table 2). At the time of submission of this progress report, all of the mice in Groups I & II (*BRCA1*$^{LoxP/LoxP}$ and *BRCA1*$^{LoxP/LoxP}$/*p53*$^{LoxP/LoxP}$) were euthanized and evaluated for the presence of neoplastic lesions. A total of 31 mice in Group II (*p53*$^{LoxP/LoxP}$), 9 mice in Group IV (*BRCA1*$^{LoxP/LoxP}$;*p53*$^{WT/R172H}$) and 34 mice in Group V (*BRCA1*$^{LoxP/LoxP}$;*p53*$^{loxP/R172H}$) remain alive in the study (summarized in Table 3). As the study is not complete, we have applied for an additional twelve month unfunded extension of this grant proposal. The incidence of tumor formation in the remaining mice and full characterization of all neoplasms will be completed during this period.

Table 2. Summary of mice evaluated (including necropsy and histopathological evaluation of tissues).

	Group I	Group II	Group III	Group IV	Group V
Injection	**BRCA1$^{LoxP/LoxP}$**	**p53$^{LoxP/LoxP}$**	**BRCA1$^{LoxP/LoxP}$ p53$^{LoxP/LoxP}$**	**BRCA1$^{LoxP/LoxP}$ p53$^{WT/R172H}$**	**BRCA1$^{LoxP/LoxP}$ p53$^{LoxP/R172H}$**
Bilateral Ad5-CMV-Cre	**41**	**11**	**36**	**32**	**4**
Single ovary Ad5-CMV-Cre	**5**	**6**	**6**	**4**	**0**
PBS	**6**	**4**	**5**	**1**	**0**

Table 3. Summary of remaining living mice on study.

	Group I	Group II	Group III	Group IV	Group V
Injection	**BRCA1$^{LoxP/LoxP}$**	**p53$^{LoxP/LoxP}$**	**BRCA1$^{LoxP/LoxP}$ p53$^{LoxP/LoxP}$**	**BRCA1$^{LoxP/LoxP}$ p53$^{WT/R172H}$**	**BRCA1$^{LoxP/LoxP}$ p53$^{LoxP/R172H}$**
Bilateral Ad5-CMV-Cre	**0**	**29**	**0**	**3**	**22**
Single ovary Ad5-CMV-Cre	**0**	**0**	**0**	**1**	**6**
PBS	**0**	**2**	**0**	**5**	**6**

Of the total 161 mice evaluated, a number of neoplasms were observed and are summarized in Table 4 (Ad5-CMV-Cre injected mice) and Table 5 (PBS injected mice). Mice in Group I in which the *BRCA1* gene was inactivated by Ad5-CMV-Cre injection developed neoplasms with a frequency of ~20% (9/42 mice). Only one ovarian neoplasm was identified and that was hilar cell tumor of the ovary. Two animals had large masses over the front paw/shoulder and the tumors were identified as adenocarcinomas. Adenocarcinoma of the lung was observed in 4/46 or 8.7 % of the Ad5-Cre-CMV injected mice and in 1/6 or 16.7 % of the PBS control injected mice. The shoulder and lung tumors in these mice were manually microdissected and subjected to genotyping analysis to confirm whether the floxed sequences present in the *BRCA1* gene of these mice was excised. In none of these 7 cases were we able to demonstrate excision of the floxed sequences present in the *BRCA1* gene were excised suggesting that these tumors are not related to loss of expression of *BRCA1*. Tumors were observed in only two of 21 evaluated cases in Group II in which the *p53* gene was inactivated. As predicted, the highest frequency of tumors observed to date were in the Ad5-CMV-Cre injected mice in Group III in which both the *BRCA1* and/or *p53* genes were inactivated. Tumors were observed in 52% (22/42) mice in this group. Ovarian tumors were observed in 24% (10/42) of these mice. Surprisingly, we did not observe epithelial ovarian tumors in these mice; rather, the ovarian tumors were poorly differentiated leiomyosarcoma of the ovary. In three mice, a preliminary diagnosis of adenocarcinoma of the fallopian tube was made and will be further evaluated by microscopic examination of additional H&E stained sections and immunohistochemical stains of various cell differentiation markers as described above. Other tumors observed in this group were primarily (21%, 9/24 cases) poorly differentiated non-ovarian peritoneal tumors without distinct morphologic or immunohistochemical features. In Group IV, in mice harboring homozygous floxed *BRCA1* gene, one wild-type and one copy of a gain of function hot spot mutation *p53*, neoplasms were observed in 28% (10/36) mice, but none were ovarian tumors. Mice harboring a single copy of this mutant p53 allele are known to develop spontaneous tumors with similar latency and

Table 4. Summary of observed pathology in mice injected with Ad5-CMV-Cre. Number indicated is the percent and the numbers in parentheses are the number of mice with the indicated neoplasm out of the total number of mice in the group.

Ad5-CMv-Cre injected mice	Group I $BRCA1^{LoxP/LoxP}$	Group II $p53^{LoxP/LoxP}$	Group III $BRCA1^{LoxP/LoxP}$ $p53^{LoxP/LoxP}$	Group IV $BRCA1^{LoxP/LoxP}$ $p53^{WT/R172H}$	Group V $BRCA1^{LoxP/LoxP}$ $p53^{LoxP/R172H}$
Mice with tumors	**20 (9/46)**	**10 (2/21)**	**52 (22/42)**	**28 (10/36)**	**75 (3/4)**
Mice with ovarian tumors	**2 (1/46)**	**5 (1/21)**	**24 (10/42)**	**0 (0/36)**	**25 (1/4)**
Ovarian leiomyosarcoma	**0 (0/46)**	**0 (0/21)**	**14 (6/42)**	**0 (0/36)**	**0 (0/4)**
Uterine leiomyosarcoma	**0 (0/46)**	**0 (0/21)**	**2 (1/42)**	**0 (0/36)**	**0 (0/4)**
Uterine and ovarian leiomyosarcoma	**0 (0/46)**	**0 (0/21)**	**10 (4/42)**	**0 (0/36)**	**0 (0/4)**
Sarcoma	**0 (0/46)**	**0 (0/21)**	**21 (9/42)**	**14 (5/36)**	**25 (1/4)**
Tubal adenocarcinoma	**0 (0/46)**	**0 (0/21)**	**7 (3/42)**	**0 (0/36)**	**0 (0/4)**
Adenocarcinoma	**13 (6/46)**	**0 (0/21)**	**0 (0/42)**	**8 (3/36)**	**0 (0/4)**
Squamous carcinoma	**2 (1/46)**	**0 (0/21)**	**0 (0/42)**	**0 (0/36)**	**0 (0/4)**
Hilar cell tumor of ovary	**2 (1/46)**	**5 (1/21)**	**0 (0/42)**	**0 (0/36)**	**0 (0/4)**
Lymphoma	**0 (0/46)**	**5 (1/21)**	**0 (0/42)**	**6 (2/36)**	**25 (1/4)**
Unclassified tumor or necrotic mass	**2 (1/46)**	**0 (0/21)**	**5 (2/42)**	**0 (0/37)**	**25 (1/4)**
Dilated endometrium	**9 (4/46)**	**24 (5/21)**	**24 (10/42)**	**3 (1/37)**	**0 (0/4)**

Table 5. Summary of observed pathology in PBS injected mice. Number indicated is the percent and the numbers in parentheses are the number of mice with the indicated neoplasm out of the total number of mice in the group.

PBS injected mice	Group I $BRCA1^{LoxP/LoxP}$	Group II $p53^{LoxP/LoxP}$	Group III $BRCA1^{LoxP/LoxP}$ $p53^{LoxP/LoxP}$	Group IV $BRCA1^{LoxP/LoxP}$ $p53^{WT/R172H}$	Group V $BRCA1^{LoxP/LoxP}$ $p53^{LoxP/R172H}$
Mice with tumors	**17 (1/6)**	**0 (0/4)**	**0 (0/5)**	**0 (0/1)**	**0 (0/0)**
Mice with ovarian tumors	**0 (0/6)**	**0 (0/4)**	**0 (0/5)**	**0 (0/1)**	**0 (0/0)**
Adenocarcinoma	**17 (1/6)**	**0 (0/4)**	**0 (0/5)**	**0 (0/1)**	**0 (0/0)**
Dilated endometrium	**0 (0/4)**	**0 (0/4)**	**20 (1/5)**	**0 (0/1)**	**0 (0/0)**

histology as tumors that arise in $p53^{+/-}$ (2). We predicted that concomitant loss of expression of *BRCA1* in the mouse ovary might result in epithelial ovarian cancers that outpaced these spontaneous tumors, but that does not seem to be the case in these mice. The majority of mice in Group V ($BRCA1^{LoxP/LoxP};p53^{loxP/R172H}$) remain alive at the time of this report. It is expected that these mice will also be susceptible to spontaneous tumor formation related to the presence of the $p53^{R172H}$ allele. Interestingly, one mouse in this group had a poorly differentiated ovarian tumor that is as of yet unclassified by tumor histology, but is positive for expression of the epithelial marker cytokeratin 8. This case is awaiting review by Dr. Ellenson.

Evaluation and analysis of the remaining mice on study will continue over the next twelve months. All tumors will be analyzed by microscopic evaluation of H&E stained tissue sections as well as tissue sections stained with various specific markers of cellular differentiation (e.g., cytokeratin 8, cytokeratin 19, CD3, CD45 and α-smooth muscle actin) and by PCR genotyping to confirm the presence of absence of floxed sequences of the *BRCA1* and/or *p53* alleles.

Key Research Accomplishments:

- Completion of breeding of $BRCA1^{LoxP/LoxP}/p53^{R172H/WT}$ and $BRCA1^{LoxP/LoxP}/p53^{LoxP/R172H}$ mice required for the study
- Completion of intrabursal injections of 80 mice with either Ad5-CMV-Cre or PBS (10 $BRCA1^{LoxP/LoxP}$, 17 $p53^{LoxP/LoxP}$, 1 $BRCA1^{LoxP/LoxP}/p53^{LoxP/LoxP}$, 14 $BRCA1^{LoxP/LoxP}/p53^{R172H/WT}$ and 38 $BRCA1^{LoxP/LoxP}/p53^{LoxP/R172H}$ mice)
- Euthanasia, complete necropsy and tissue collection on 161 mice
- Histopathological evaluation of tissue specimens from a majority of these 161 mice
- Analysis of genotype of tumor tissues by PCR analysis
- Immunohistological staining of tumor specimens for expression of cellular markers of differentiation including cytokeratin 8, cytokeratin 19, CD3, CD45, α-smooth muscle actin (α-SMA).

Reportable Outcomes:

- Inactivation of conditionally expressed *loxP* flanked alleles of the *BRCA1* and/or *p53* genes in the mouse ovary by intrabursal injection of Adenovirus-Cre recombinase results in a high frequency of ovarian and non-ovarian neoplasms.
- Frequency and latency of tumor formation
- Tumor histology

Conclusions:

Inactivation of conditionally expressed *loxP* flanked alleles of the *BRCA1* and/or *p53* genes in the mouse ovary by intrabursal injection of Adenovirus-Cre recombinase results in a high frequency of ovarian and non-ovarian neoplasms. Unlike a previously reported mouse model of epithelial ovarian cancer resulting from conditional inactivation of the *p53* and *Rb* genes in the mouse ovary (1), the incidence of epithelial

ovarian carcinomas is rare in mice with conditional inactivation of both the *BRCA1* and *p53* genes. We have communicated our results with our colleagues at meetings and have learned that other groups performing similar experiments have had similar results. Although this is not the predicted outcome, the results of this study are reportable and will be submitted for publication after all study groups have been completely analyzed.

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Appendices:

None